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Effects of chronic upper respiratory bypass cannula on the morphology and function of the eye of the sheep

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EFFECTS OF CHRONIC UPPER RESPIRATORY BYPASS CANNULA ON THE MORPHOLOGY AND FUNCTION OF THE EYE OF THE SHEEP

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Effects of chronic upper respiratory bypass cannula on the morphology and function of the eye of the sheep

by

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DEDICATION

This dissertation is dedicated to the memory of my father, Boniface Ash Ohale, who ascended to the Lord during the course of this study.

INTRODUCTION

Hales (1973) has shown that metabolic heat production in the brain of a homeotherm is relatively higher than in other tissues. Further, Burger and Fuhrman (1964) and Carithers and Seagrave (1976) have demonstrated that cerebral tissue is particularly prone to damage by hyperthermia in dogs. Circulating blood normally eliminates heat continually from the brain thereby reducing the chance of overheating, since the general core temperature influences brain temperature. However, since the limit of tolerance rise in core temperature appears to be that of the brain, any means of locally cooling the brain would increase heat tolerance (Cabanac and Caputa, 1979).

Accordingly, selective cooling of the brain during heat stress has been reported by various investigators to be a device of controlling the brain temperature for animals with or without the presence of the carotid retia. Countercurrent heat exchange between the arterial and venous blood is a well-established mechanism for temperature regulation in mammals. The role of the nasal mucosa and the carotid rete or the intracavernous part of the internal carotid artery in regulating brain temperature was described by Taylor (1966) in the goat, Magilton and Swift (1967) in the dog, Baker and Hayward (1967) in the cat, and Kluger and D'Alecy (1975) in the rabbit. The functional significance of the above relationship was demonstrated by Baker and Hayward (1968b) in the sheep, Taylor (1969) in the antelope, and Magilton and Swift (1968, 1970a) in the dog. Further, in the dog, the latter authors described two physiologic heat exchange systems: (1) an "external heat exchange system" between the venous plexuses and the

ambient air over the nasal mucosa and (2) an "internal heat exchange system" between the cooled venous blood, draining from the venous plexuses of the nasal mucosa, in the cavernous sinus and the carotid rete or the intracavernous part of the internal carotid artery bathed in it. Further, Baker and Hayward (1968a, 1968b) observed that the central arterial blood of the sheep was cooled as it passed across the carotid rete in the cavernous sinus to the cerebral vessels. Besides, by both mechanical and chemical occlusion of the nostrils of the sheep, Young et al. (1976) demonstrated increases in the hypothalamic temperature and concluded that evaporation from the upper respiratory tract has an immediate as well as a local effect on brain temperature. In effect, cooled venous blood from the nasal mucosa is returned to the heart, under normal physiologic conditions, through the cavernous sinus, where countercurrent heat exchange between it and the relatively warm core temperature blood in the internal carotid artery takes place.

A more permanent occlusion of the nostrils has been achieved by recent investigators. For example, Baker et al. (1974) reported the effects of tracheostomy on the increase in brain temperature when the dog breathed through the tracheal opening. By chronic implantation of a reversible tracheal bypass cannula, Kluger and D'Alecy (1975) recorded increases in hypothalamic temperature in rabbit during bypass breathing. This finding was attributed to the functional elimination of the "external heat exchange system" of Magilton and Swift (1968, 1970b), which was the source of the cooled venous blood for the "internal heat exchange system" within the cavernous sinus, as previously mentioned. The above experiments, in a way, simulate a condition observed in mongoloids in which

respiration is largely accomplished through the mouth due to significantly underdeveloped facial bones (Benda, 1946; Apgar, 1970).

Some researchers have reported a linear correlation between cerebral vasodilatation, increased blood volume, and increased cerebrospinal fluid pressure. Abrams et al. (1965) stated that the rate of heat transfer from a heat producing mass (hypothalamus) to a coolant fluid (arterial blood) must depend, in part, on the rate of flow of the coolant fluid through the heated mass. Besides, Hayward and Baker (1969) observed, with 10% carbon dioxide breathing in monkeys, a cerebral vasodilatation as well as an increased blood flow through the brain. This increased flow was concurrent with a decrease in brain temperature since metabolic heat was quickly removed from the site of its production. In addition, Forbes and Wolff (1928) described parallels between changes in the cerebrospinal fluid pressure and the diameter of the pial artery in primates. Sawada and Tazaki (1977), in primates, concluded that decreases and increases in the cerebrospinal fluid pressure were related to cerebral vasoconstriction and vasodilatation, respectively.

The origin of the retina of the eye has been traced to the primitive fore-brain (Arey, 1974) and its continued similarity to the central nervous system has been documented by Cogan (1974). The retinal arteries arise either entirely (man) or partly (sheep) from the same source that supplies the brain (cerebral arterial circle). However, common to both blood supplies to these structures is the fact that they pass through either the ophthalmic plexus and/or the cavernous sinus, both of which receive cool venous blood from the nasal mucosa (Getty, 1975; Carpenter, 1976). Further, prolongations of the cerebral meninges form the sheath of the optic nerve

extracranially. The dura of the optic nerve is fused to the periosteum of the optic canal, but its subarachnoid space communicates with the intracranial subarachnoid space containing cerebrospinal fluid (Glaser, 1978). Further, Tenner and Trockel (1968) demonstrated the rare occurrence of free passage of intracranial subarachnoid air into the orbital portions of the optic nerve.

A very complex relationship exists between the eye and cerebral vascular conditions. For instance, changes in intracranial and cerebrospinal fluid pressures have been shown to affect the functional morphology of the eye in rhesus monkeys (Tso and Hayreh, 1977). Efficient blood flow through the retina is not only important for its visionary function, but also due to irreversible changes that might occur from an overheated nervous tissue (Minard and Copman, 1963). Besides, Ffytche (1974) reported an inverse relationship between the retinal blood flow and the intraocular pressure in animals, i.e., a progressive decrease in the blood flow velocity as the intraocular pressure increased. Recent work has also demonstrated the effects of temperature on the functional efficiency of the retina. Winkler (1972), working on isolated rat retina, observed increases in the amplitude and rate of rise of the components of the electroretinogram (ERG) with corresponding increases in the temperature of the incubating medium. He further noted that in temperatures below $10^{o}C$, the ERG was entirely abolished. Moreover, O'Day and Young (1979) have mentioned changes in the length of the outer segment of the visual cells (i.e., in the rods and cones) in response to warm and cold conditions in the goldfish. It can be inferred from these results that the retina functions optimally within some specific temperature ranges.

This experiment is designed to answer the following questions, when the sheep is placed on an upper respiratory bypass breathing:

1) Are there any changes in:
   (a) the retinal vasculature?
   (b) the electrical activity of the retina?
   (c) the arterial Pco_2, Po_2, and pH?
   (d) the intraocular pressure?
   (e) the functional morphology of the optic nerve head and the retinal layers?
2) Are the changes observed, if any, similar to those eye conditions reported in mongoloids (Down's syndrome in man)?

LITERATURE REVIEW

Many investigators have demonstrated the possible role of the nasal mucosa in thermoregulation of the brain in mammals, which led to an extensive study of the complex vascular pattern of the nasal mucosa. For example, Swindle (1937) reported that in the rabbit, dog, sheep, and deer, the nasal blood vessels are arranged in superficial, middle, and deep layers. Further, Scott (1954) compared the surface area of the nasal mucosa of various animals and noted that in the seal it may approach, if not exceed, the total skin surface area due to the complexity of the conchae. But, in the sheep, pig, deer, horse, kangaroo, lemur, and new world monkey with a less developed concha, this deficiency is compensated by the high vascular complexity of the nasal mucosa, especially in the ungulates. He also stressed the importance of the nasal vasculature in temperature regulation, especially in animals covered by wool, feathers, or thick hair, which he claimed were a means of conserving and not losing heat. He further hypothesized that in wooled sheep the nasal vasculature might be very effective in heat loss, thus enabling the animal to survive in both hot and cold weather. Dawes and Prichard (1953) demonstrated in the sheep, pig, and goat that the latticework arteries run obliquely in the nasal septum and horizontally and much closer together in the dorsal concha. They further investigated the significance of the nasal arteriovenous anastomoses of the dog, especially at the nasal tip and ventral nasal concha, and concluded that anastomoses were more numerous in those areas where the mucosa was directly exposed to the inspired air. Naessen (1970) observed that the respiratory epithelium of the nasal cavity of

man and guinea pig possesses furrows or gutters aligned in rows and postulated that the additional surface area provided by these furrows might be essential in heat transfer.

Ralston and Kerr (1945) observed in humans a fall and rise of nasal temperature after general chilling and warming of body temperature, respectively. Besides, Cauna (1970) reported the presence of large fenestrated subepithelial capillaries in the mucosa of the lateral nasal wall of man. These fenestrations were concentrated over the superficial aspect of the endothelial tube which adjoins the ciliated epithelium. In addition, he mentioned that the deeper portion of the capillary wall had a well-developed layer of pericytes and the enclosing basement membrane frequently containing fine pores. The endothelial cells of the capillaries around the nasal glands were also found to have fenestrae. Further, Dixon et al. (1949) and Scott (1954) demonstrated that in tracheostomized patients, the nasal cavity or nose temperature was constantly higher by 1.2°C than the normal. Krabill (1979), by placing a bead thermistor in the nasal vestibule of the unanaesthetized sheep, observed an increase of 6°C of the nasal mucosa during bypass breathing. The above authors attributed this variation to the absence of heat exchange in the nasal mucosa. Besides, Negus (1958) noted that most animals pant with their mouths closed because of the morphologic arrangement of the epiglottis above the soft palate preventing mouth breathing. Further, Blatt et al. (1972) ascribed the lateral nasal glands (Steno's gland), opening inside the nasal vestibule of the dog, as the source of water for evaporative cooling of the nasal mucosa and observed that evaporative heat loss increased as secretions from the glands increased. They concluded that

these glands provide a mechanism for heat loss from the venous plexus in the nasal mucosa when the ambient temperature is higher than the temperature of the blood, since evaporation removes a considerable amount of heat. Besides, Baker (1972) has shown that the heat loss in the nasal mucosa of the cat depends on the rate of air flow as well as blood flow through the mucosal surfaces. In a cool environment, when the respiratory rate is relatively constant, vasoconstriction of the mucosal vessels decreases the nasal heat loss and vasodilatation increases it. Thermocouples implanted in the nostrils of the sheep and cat showed an increase in temperature of the nasal mucosa with increase in blood flow and a corresponding decrease in temperature with decrease in blood flow.

Magilton and Swift (1969), by irrigating the alar fold of the nasal cavity of the dog with cold and warm water, observed corresponding decrease and increase in the temperature of the angularis oculi and facial veins. They noted that the slope of the temperature curves between the angularis oculi and facial veins of the same side varied from one trial (hot water to cold water and back to hot water irrigation) to the other during the same experiment. Baker (1979) traced the flow of the venous blood from the nasal mucosa by injecting colored latex into the nose of preserved sheep head and observed that the latex filled the cavernous sinus at the base of the brain containing the carotid rete (rete mirabile epidurale rostrale). Khamas[1] (1979) has shown by venography in anaesthetized sheep unilateral filling of the cavernous sinus at the base of the brain.

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[1]Wael Khamas, graduate student, Department of Veterinary Anatomy, College of Veterinary Medicine, Iowa State University, Ames, Iowa. (Private communication).

Besides, Magilton and Swift (1967) postulated that the usual drainage of the nasal mucosa was through the angularis oculi -- ophthalmic vein to the cavernous sinus in the dog.

Baker (1979) cited Herophilus, 300 B.C., as the first to observe a small plexus of vessels at the base of the brain. This plexus was later described by Galen about the middle of the second century A.D., who referred to it as a wonderful rete. Tandlar (1899) studied the arteries of the head, including the carotid rete, in many species. Daniel et al. (1953) described the rete in the cat, sheep, goat, ox, and pig, and a rudimentary form in the dog. In all these species, the rete lies within the cranium, bathed in venous blood in the cavernous sinus, except in the cat, where it lies extracranially. No rete has been described for the rabbit, rat, monkey, and man. Further, Baker (1979) described this network in the cat as consisting of medium sized vessels (about 200-300μM in diameter). She observed large reservoirs of venous blood at the base of the brain, where the arteries that supply the brain enter the cranial cavity. This basic pattern is characteristic to all animals with or without the carotid rete. In animals without a rete, the internal carotid artery passes through the cavernous sinus and, on emerging, forms the cerebral arterial circle.

Countercurrent heat exchange between the arterial blood in the carotid rete or the intracavernous portion of the internal carotid artery and the venous blood in the cavernous sinus, in the various domestic mammals has been of profound interest to many investigators. For example, Magilton and Swift (1968) demonstrated the occurrence and function of two physiologic heat exchange systems in the head of the dog, which under normal

physiologic conditions aid to cool the brain. Their experiments verified the presence of both an "external" (between the venous plexus of the nasal mucosa and the ambient air) and an "internal" (between the arterial blood in the carotid rete and venous blood in the cavernous sinus) heat exchange mechanisms. They implanted thermistors (bead and needle) adjacent to the right and left angularis oculi veins and in the pons, cerebral peduncle, hypothalamus, thalamus and cerebral cortex. On irrigation of the alar fold of the ventral nasal concha with cold and warm water, they observed that at all the thermistor locations, the temperature of the brain tissue varied in the same direction as that of the angularis oculi vein and of the irrigating water. Further, Baker and Hayward (1968b) blew cold air into the nostrils of the sheep at the rate of 50 times per minute for five minutes and noted a temperature decrease of $2^{\circ}C$ in the venous blood of the cavernous sinus. They inferred the possibility of heat transfer from the relatively warm arterial blood passing through the carotid rete to the relatively cool venous blood, draining from the nasal surfaces, surrounding the rete inside the cavernous sinus. Thus, they concluded that heat exchange between the central arterial blood in the carotid rete and the cranial venous blood in the cavernous sinus is the major factor regulating cerebral arterial blood and brain temperature in the sheep. Hemingway et al. (1966) observed that the hypothalamic temperature in the sheep is cooler than the normal body temperature under normal physiologic conditions, while Hellstrom and Hammel (1967) hypothesized that the rate of respiration in the dog and other panting animals is strongly dependent upon both hypothalamic and ambient air temperatures. These two factors will correspond to two heat exchange systems described

by Magilton and Swift (1967) in the dog. Further, Baker and Hayward (1968b) have attributed the cooling of the central arterial blood in the carotid rete as a protective mechanism, which enables the sheep to cool its brain temperature in heat stress conditions and, therefore, is a means of preventing cerebral overheating, while Rawson (1976) claimed that the above characteristics are responsible for the high heat tolerance of the sheep.

In addition, Robertshaw (1976) observed that ruminants pant with their mouths closed and that heat exchange must, therefore, take place at the nasal mucosa. Indirect evidence for this was obtained from the work of Bligh (1957) in the calf, who demonstrated that there is no change in the temperature of blood as it passes through the lungs during panting, while there is a considerable cooling of the blood draining the head (Ingram and Whittow, 1962). Then, he concluded that cool blood from the nasal passages enters the cavernous sinus and that since the arterial supply to the head also passes through the same venous sinus, arborizing into a rete of vessels, this arrangement allows the exchange of heat between the two blood streams flowing in opposite directions, which are in effect a countercurrent heat exchanger. Baker and Hayward (1968a) noted that in the resting sheep, shifts in hypothalamic and other brain temperatures paralleled temperature shifts in the cerebral arterial blood, which was cooler than central arterial blood. Later, they (1968d) demonstrated, in a recumbent sheep at 20°C, that the cerebral arterial blood was 0.5°C cooler than carotid blood. During periods of arousal and paradoxical sleep, vasoconstriction of the nasal mucosa and the ear skin occurred, and

the temperatures of the cerebral arterial blood and brain rose without comparable rise in central arterial blood temperature (Baker and Hayward, 1968c).

In the gazelle, Taylor (1969) reported that the hypothalamus was as much as 2.9°C cooler than carotid arterial blood and attributed this finding to the cool venous blood from the nasal passages draining into the cavernous sinus. Further, Taylor and Lyman (1972) observed that in running antelopes, the brain temperatures rose more slowly than rectal and carotid arterial temperatures. After 5 minutes of running at a rate of 40 km per hour, they noticed that the brain was 2.7°C cooler than the blood in the carotid artery. They concluded that blood supplying the brain was cooled via a countercurrent heat exchange with cool blood draining the nasal mucosa (in the cavernous sinus). Besides, a similar investigation by Baker (1979), in exercising dogs, showed that the dogs maintained their brain temperature more than 1°C below the temperature of the blood leaving the heart. She also measured the carotid blood flow and noted that it increased gradually during exercise and remained elevated for the duration of the exercise. She concluded that the brain cooling, which occurred in these dogs, is probably due to acceleration of heat exchange between the cerebral arterial blood and venous blood draining the upper respiratory tract and that a large proportion of carotid flow during exercise must be destined for the nasal passages, since maximum flow to the tongue was small as compared to the flow they observed.

Occlusion of normal nasal breathing by various methods has been observed to have varying effects on the temperature of the cerebral arterial blood and the brain. For example, Baker et al. (1974), by blocking the

air flow through the nostrils of the sheep, noted an increase in hypothalamic temperature as well as a differential between the common carotid temperature and that of the hypothalamus, but when the animal was allowed to breathe normally, the hypothalamic temperature decreased. The maintained differential was attributed to the functional elimination of the evaporative cooling in the nasal passages and reduced heat loss from the carotid rete to the now fairly warm blood in the cavernous sinus. Further, Kluger and D'Alecy (1975) demonstrated that an open reversible tracheal bypass cannula, surgically implanted in the rabbit, caused air flow to cease in the nasal passages, resulting in an increase in hypothalamic temperature; with the bypass closed, normal air flow through the nostrils resumed and consequently there was a decrease in hypothalamic temperature. During normal breathing, however, the hypothalamic temperature was lower than rectal temperature, but with the bypass open, the hypothalamic temperature rose to the same level as rectal temperature. They concluded that even in animals, such as the rabbit, without a carotid rete the hypothalamic temperature is influenced by upper respiratory cooling of venous blood and that the ensuing transfer of heat from the warmer internal carotid arterial blood to the cooler venous blood in the cavernous sinus can effectively cool the brain. Employing a similar setup for the sheep, Krabill (1979) recorded an average increase of 0.37^{o}C in brain temperature during bypass breathing.

The carotid rete or rete mirabile epidurale of the sheep (International Committee for Veterinary Anatomical Nomenclature, N.A.V., 1973), consists of a compact network of blood vessels, lying epidurally in the cavernous sinus at the base of the brain, having two halves with poorly

developed rostral and caudal intercommunicating arteries. The rete extends from the foramen orbitorotundum, rostrally, to just beyond the foramen ovale, caudally, and incompletely invests the hypophysis cerebri (pituitary gland). The rete is bathed in a venous lake of blood draining from areas outside the cranial cavity, especially from the nasal and facial areas. Blood destined for the more dorsally located cerebral arterial circle (from which most areas of the brain receive their blood supply) must at first pass through this venous lake via the carotid rete (Daniel et al., 1953; Baldwin and Bell, 1963; Baldwin, 1964; Getty, 1975).

The rete essentially receives afferent supply from two branches off the maxillary artery: the rostral rete branches and the caudal rete branch. These branches enter the cavernous sinus via the foramen orbitorotundum and foramen ovale, respectively, and by repeated branching form the carotid rete. In young animals, the internal carotid makes an insignificant contribution. However, the presence of the extracranial part of the internal carotid artery has been described for sheep up to nine months (Baldwin, 1964). When present, the artery retains its integrity inside the rete as it courses through it. It receives numerous branches from the rete before finally emerging as the efferent artery from the dorsomedial aspect of the rete. It pierces the dura mater and divides to form the cerebral arterial circle (Baldwin, 1964; Getty, 1975). Daniel et al. (1953) in the ox and sheep, Anderson and Jewell (1956) in the goat, and Baldwin (1964) in the sheep reported that the basilar artery in ruminants is reduced to a minor branch of the carotid arterial circle lacking any significant connection with the vertebral arteries. Baldwin (1964), however, stated that the sheep differs from the ox in the

arrangement of the cranial arteries, a fact which both Tandlar (1899) and Ask-Upmark (1935) have seemingly overlooked. He reported the absence of a basioccipital plexus contributing to the rete as in the ox, since there is no caudal rete mirabile in the sheep. Thus, he concluded that the blood supply to the brain of the sheep is through the rostral and caudal rete branches off the maxillary artery with an insignificant contribution from the internal carotid in fetal lambs and lambs up to nine months old. However, contributions of the branches arising from the external ophthalmic artery to the rete have been described (Zietzschmann, 1912; Heeschen, 1958; and Nickel and Schwarz, 1963). In the sheep, all blood passing to the brain via the cerebral arterial circle goes through this countercurrent heat exchange system between blood in the carotid rete and the venous blood in the cavernous sinus and is, therefore, temperature-conditioned.

The blood supply to the retina arises from two sources in the sheep: the external ophthalmic artery off the maxillary and the internal ophthalmic off the intracranial portion of the internal carotid artery. The external ophthalmic pierces the periorbita and arborizes into a compact and interlacing network of small arteries - the ophthalmic rete beneath the dorsal rectus muscle (Prince et al., 1960). The ophthalmic rete is surrounded by the ophthalmic venous plexus, which receives a considerable amount of blood from the dorsal nasal vein draining the nasal area and represents a route for blood from the coolest portions of the nose (Schmidth-Nielson et al., 1970). Further, Carlton and McKean (1977) noted that in the pronghorn (a ruminant) the ophthalmic plexus also receives blood from the rostral auricular vein, which joins the transverse facial to form the superficial temperal vein. They claimed that the ophthalmic rete

is embedded in venous blood with the veins and arteries arranged to accomodate blood flow in opposite directions and further concluded that such arrangement has the necessary anatomic requirements expected of a countercurrent heat exchanger. This observation might indicate that blood to the eye is also temperature-conditioned since the internal ophthalmic artery (arising from the intracranial part of the internal carotid artery) has passed through the "internal heat exchanger" of Magilton and Swift described previously.

The Retina: Analogy with the Nervous System

Arey (1974) and Carpenter (1976) described the retina as the evaginated portion of the primitive forebrain, the optic pouch, which secondarily is invaginated to form the two layered optic cup. From the inner layer of the cup (neural portion of the retina) differentiates the receptor rod and cone cells, the horizontal and bipolar neurons, and the multipolar ganglionic cells, whose axons form the optic nerve. This inner layer forms a fiber tract between two parts of the brain. Cogan (1974) described the retina as an ectopic portion of the brain which never does lose its similarity to the central nervous system. He observed that the inner layers of the retina show a lamination comparable to that of the gray matter in the cortex, while the optic nerves exhibit the same compartmentalization by pia as well as the absence of Schwann cells as characterized by white matter in the brain. The rods and cones (neuroectodermal layer of the retina) were compared to the sensory receptors of the skin and the bipolar cells (forming the middle cellular laminar layer of the retina), which relay neural impulses from the rods and cones, to

the dorsal root ganglion cells in the rest of the sensory nervous system. Further, he claimed that the ganglion cells were analogous to the neurons having their cell bodies in the gray matter of the spinal cord and primary sensory nuclei in the brain stem, while the optic nerve and tract were referred to as the photoconductive counterpart of the sensory tracts within the spinal cord and lemnisci of the brain stem. He concluded that although the optic nerves are designated as nerves, they are more analogous to the tracts of the central nervous system than to peripheral nerves. This analogy of the optic nerve to peripheral nerve becomes more apparent clinically due to its lack of regenerative capacity and its extreme susceptibility to certain demyelinative diseases. Anderson (1969a, 1969b, 1970a, 1970b) noted that oligodendrocytes constitute about two-thirds of the interstitial cells of the retrolaminar portion of the optic nerve axons in primates where they are responsible for myelin formation and also concluded that the optic nerve was more of a white fiber tract of the brain.

In the optic canal of man, the dura mater around the optic nerve and the periosteum are fused and its subarachnoid space is continuous with the intracranial subarachnoid space, containing the cerebrospinal fluid (Glaser, 1978). Besides, the rare occurrence of free passage of subarachnoid air into the orbital portions of the optic nerve has been demonstrated by Tenner and Trockel (1968) in primates. Other studies have revealed not only the free passage of diaminoacridine dyes, horseradish peroxidase, and sodium fluorescein from the intracranial to the orbital subarachnoid space, but also the free permeability of these substances into the pia of the optic nerve. For example, Rodriguez-Peralta (1963)

injected diaminoacridine dye into the cisterna magna of rabbits, cats, guinea pigs, and rhesus monkeys and, after one hour, observed a high concentration of the dye in the subarachnoid space of the optic nerve as well as diffusion into the optic nerve, and optic nerve head, including the intermediary tissue of Kuhnt. Similar observations were made by Tsukahara and Yamashita (1975) when they injected horseradish peroxidase in the lateral ventricles of the mice. This free passage and diffusion of cerebrospinal fluid from the intracranial subarachnoid to the orbital part may have some influence on the optic nerve pressure up to the area behind the area cribrosa of the sclera, a factor which Hayreh (1978) claimed to play a role in the pathogenesis of edema of the optic nerve head in primates.

MATERIALS AND METHODS

This study is a part of an overall project entitled: Development of an animal model for studying the effects of interrupting the normal brain temperature regulation mechanism on the plasma pituitary hormone level(s) and the probability of eye defects in the sheep, whose various facets are currently being investigated in the Department of Veterinary Anatomy, College of Veterinary Medicine, Iowa State University, Ames, Iowa.

Thirty eight sheep (12 to 50 kg body weight), of various ages ranging from 8 weeks to 5 years, were used for this study (Table 1). Prior to the investigation, each animal was sheared and housed in an animal room for three days (temperature, 60° to 61°C) for acclimatization. During this period, the rectal temperature, respiratory rate, and heart rate were recorded thrice daily at 8 a.m., 12 noon, and 4 p.m. This regime was considered necessary for subsequent handling and management of the animals during the course of experiments and to protect them from unforseen environmental influences.

Tracheal Bypass Implantation

Following three days acclimatization, a modified reversible tracheal bypass cannula was chronically implanted in each animal (Figure 1), following standard surgical procedure. This device was, at first, designed and described by Kluger and D'Alecy (1975) for rabbits. Atropine (¼ mg/kg body weight) was administered subcutaneously to each sheep, 10 to 15 minutes prior to the induction of fluothane (Halothane, U.S.P. Veterinary Medical Division, Ayerst Laboratories, Inc., N.Y., New York) anaesthesia.

Table 1. Data of animals used for the investigations

Serial No.	Sheep No.	Breed	Weight (Kg)	Age in Yrs.
1	172	Rambouillet	52	5
2	1728	Rambouillet	45	3
3	195	Rambouillet	35	1
4	197	Rambouillet	35	1
5	1978	Rambouillet	35	1
6	001	Suffolk	14	8[a]
7	185	Rambouillet	35	1
8	120	Rambouillet	30	1
9	146	Rambouillet	35	1
10	42	Suffolk	12	10[a]
11	105[b]	Rambouillet	30	1
12	51	Rambouillet	30	1
13	60	Rambouillet	30	1
14	84	Suffolk	14	8[a]
15	39	Rambouillet	40	1
16	133	Rambouillet	35	1
17	31	Rambouillet	35	2
18	121	Rambouillet	30	2
19	70	Rambouillet	30	2
20	158	Rambouillet	30	3
21	316	Rambouillet	35	2
22	210	Rambouillet	30	2

[a]Weeks.
[b]Same as for Serial No. 27.

Table 1. *Continued*

Serial No.	Sheep No.	Breed	Weight (Kg)	Age in Yrs.
23	214	Rambouillet	30	1.5
24	270	Rambouillet	40	2.5
25	290	Rambouillet	30	2
26	262	Rambouillet	30	2
27	105	Rambouillet	30	1
28	174	Rambouillet	35	2
29	47	Rambouillet	40	3
30	201	Rambouillet	40	3
31	43	Suffolk	14	10[a]
32	128	Suffolk	12	8[a]
33	169	Rambouillet	35	2
34	143	Rambouillet	35	2
35	181	Rambouillet	40	3
36	701	Rambouillet	30	1
37	725	Rambouillet	30	1
38	716	Rambouillet	30	1
39	734	Rambouillet	30	1.5

A midventral incision was made at the caudal 1/3 of the neck, and, by blunt dissection, the recurrent laryngeal nerves were carefully separated from the exposed trachea. Then, the trachea was transected and each cut end was held with an Allis forceps. The cranial and caudal tubal extensions of the cannula (Figure 1) were immediately inserted into the cranial and caudal segments of the trachea, respectively. Both tracheal segments were secured on the tubal extensions of the cannula by using a surgeon's knot and a #2 surgical silk. The ends of the surgical silk were then looped across the base of the body of the cannula and tied off. Additional supporting sutures were placed on each side of the cannula across the cut end of the trachea. This resulted in an airtight seal and an uninterrupted air flow from the nostrils to the lungs. In some animals, it was necessary to remove 2 to 3 cartilaginous rings before inserting the cannula, to avoid crimping of the trachea when the animal extended its neck. The incision was closed, leaving the body of the cannula projecting for about one inch from the skin surface and a purse-string suture was placed in the skin around its base. Penicillin and dihydrostreptomycin (Combiotic, Pfizer, Inc., New York, N.Y.) were administered (50 mg/kg body weight) and continued throughout the period of the investigation to counter any local infections. A three-day postsurgical recovery period was allowed prior to any investigative procedures.

Retinal Vasculature: Fundic Photographs

This segment of the investigation was designed in three phases as follows:

Prebypass phase (N1): This was defined as the 4th-6th postoperative days. During this phase, the flow-through insert of the cannula was in place (Figure 1) and breathing was normal through the nostrils.

Bypass phase (B): This phase includes the 7th-9th postoperative days. The flow-through insert was replaced by the bypass insert of the cannula and breathing was accomplished through the tracheal opening (i.e., normal nasal breathing was interrupted).

Postbypass (N2): This is the 10th-12th postoperative days. The flow-through insert was reestablished, resuming normal nasal breathing.

Fundic photographs were taken from 14 sheep (Table 1: #1-14). The pupils were dilated with 1% Tropicamide, an ophthalmic solution (Mydriacyl; Alcon Laboratories, Inc., Fort Worth, Texas). A portable Nikon fundic camera attached to an ophthalmoscope, a strobe light and base unit was used for all fundic photographs. Each sheep's eye was illuminated by a viewing light with an adjustable light intensity. The magnification and focus were preset and the strobe intensity was set between 1 and 6. The eyelids were held open with the index finger and thumb of the left hand and the camera moved up and down until the retinal vessels came into focus. Five photographs were taken of areas around the optic disc on the last day of each phase (i.e., on the 6th, 9th, and 12th postoperative days), using Kodachrome ASA 64, KR. 135-20 slide film (Eastman Kodak Company, Rochester, New York). The best three fundic photographs from each phase were selected for comparison.

The outer diameter of the superotemporal retinal vein was measured in each of the selected slides at a distance approximately 1 cm

from the margin of the optic disc (Figure 2). A stereozoom dissecting scope (Bausch and Lomb, Inc., Rochester, N.Y.) and a micrometer eyepiece were used for all measurements. The distance between the objective lens and the 2x2" slides was kept constant throughout the measurements.

Electrical Activity of the Retina

The prebypass phase in this setup was described as the last three minutes of a ten minute interval in which the flow-through insert of the cannula was in place, while the bypass phase was the last three minutes of a ten minute interval following the prebypass phase in which the bypass insert was in place. During the interval following each phase, the corresponding inserts were interchanged and the upper respiratory tract was cleared of any mucus.

All recordings were made in a soundproof electrically-shielded darkroom. Each sheep was dark-adapted for three minutes prior to each recording. Each of the five sheep used for this study (Table 1, #15-19) had two trial periods, two days apart. During each trial, the electroretinogram (ERG) was recorded for both phases three times and photographed on the same polaroid film (Type 47, Polaroid Corporation, Cambridge, Mass.) using a C59 oscilloscope camera (Tetronix, Inc., Beaverton, Oregon).

Each sheep was premedicated with atropine (¼ mg/kg body weight) prior to the induction of fluothane anaesthesia. The sheep was placed in right lateral recumbency on a wooden table in the soundproof darkroom and the head was supported to hold it steady. The pupils were maximally dilated with 1% Tropicamide, ophthalmic solution. Then, a cup electrode was filled with a balanced salt solution (B.S.S.; Alcon

Laboratories, Fort Worth, Texas) and carefully placed over the cornea. One E2B subdermal electrode (Grass Instrument Co., Quincy, Mass.), the negative electrode, was placed about 3 cm from the lateral canthus of the eye, while another E2B electrode, the ground, was placed over the masseter muscle. The electrodes were connected to a preamplifier P511J (Grass Instrument Co., Quincy, Mass.) and the capacitance coupled to a single beam, dual trace storage oscilloscope D11 (Tetronix, Inc., Beaverton, Oregon). The ERG was displayed on the oscilloscope screen. The ERGs were evoked by the use of a photostimulator, PS 22A (Grass Instrument Co., Quincy, Mass.) emitting flashes at the rate of one flash per second. The light source was 8 cm from the cornea. The stimulus monitor was used to calibrate the recordings. The amplitude and duration of the b-waves for both phases were measured and statistically analyzed.

Blood Gas Analysis

A similar experimental design to that used for the ERG recordings was adopted for this study (Table 1: #20-24).

The common carotid artery was cannulated with a catheter connected to a three way valve. Heparinized saline (3000 i.u) was injected into the catheter. Heparinized 5 ml syringes with fitting needles and caps were used to collect blood samples during the corresponding phases. The blood samples were placed in a beaker containing ice chips and analyzed on a Blood Gas Analyzer, 513 (Instrumentation Laboratories, Lexington, Mass.). The gas analyzer was calibrated depending on the barometric pressure at the time of analysis. The partial carbon dioxide and oxygen tension pressures were calculated and statistically compared during nasal and bypass breathing.

Intraocular Pressure

The prebypass recordings were taken during the last five minutes of a ten minute interval in which the flow-through insert of the cannula was in place, while the bypass recordings were made in the last five minutes of a ten minute interval following the prebypass phase with the bypass insert in place. The inserts were interchanged during the intervals following each phase and the upper respiratory tract cleared of any mucus.

Five sheep (Table 1: #25-29) were anaesthetized with fluothane and the pupils were dilated with 1% Tropicamide, ophthalmic solution. Each sheep was heparinized (3000 i.u), and the anterior chamber of the eye was cannulated with a modified 23 gauge hypodermic needle connected via a heparinized saline-filled tube (Polyethylene, #10) to a low pressure transducer, P22 (Statham, Inc., Puerto Rico) and on to a Beckman R611 pen recorder (Beckman Instruments, Inc., Schiller Park, Ill.) to measure intraocular pressure from each eye. The common carotid artery on one side was exposed and cannulated with a fine polyethylene catheter which was previously heparinized. The catheter was then connected to another P22 transducer and again to the Beckman pen recorder to measure the systemic pressure. The pen recorder was precalibrated for both the intraocular and systemic pressures.

Histologic Study

Histologic sections of the retina and optic nerve head were made of 22 eyes from animals which had been on bypass for at least six days during the course of the study and another 10 from animals, which had the cannula implanted but had never been on bypass, which served as controls.

Each sheep was euthanatized with sodium pentobarbital and the external jugular veins were immediately severed to preclude excessive bleeding in the orbits during enucleation. The eyelids were held together with Allis forceps and skin incisions were made around the margins of the bony orbit. A curved blunt scissors was inserted through the incision and worked around the bony orbit to sever the ocular adnexa. The excised globe was quickly cleaned of attaching extraocular tissues and immersed in 500 ml of Zenker's glacial acetic acid for fixation.

Fixation and processing

Zenker's fluid:

Distilled water	1000 ml
Mercuric chloride	50 gm
Potassium dichromate.	25 gm
Sodium sulphate	10 gm

Five millimeters of glacial acetic acid was added for each 100 cc of Zenker's fluid before use. Fixation was for 24 hours in a cold room (temperature, 25°F).

Following fixation, each eye was washed under cold running tap water to remove excess acid for 24 hours after which it was immersed into 500 ml of 70% ethanol for another 24 hours for hardening. Two calottes were removed by two parasagittal incisions from the corneoscleral junction using a new double-edged razor blade for each eye. The lens and the cornea were then removed and the rest of the globe was passaged as follows through graded alcohol and clearing agents.

Ethanol (80%)	2 hours
" (95%)	2 "
" (95%)	2 "
" (Absolute)	2 "
" (")	2 "
Xylene and absolute Ethanol (50/50)	2 "
Chloroform	2 "
"	4 "
Xylene	1 hour
"	1 "
Tissue-Prep[1] bath	12 hours
"	2 "

The passage from the xylene to the Tissue-Prep bath was carried out in a vacuum oven (temperature, 61°C) with a vacuum pressure of 15 mm Hg. A rotary microtome was used to cut sections seven micrometers thick.

The sections were deparaffinized and mercuric chloride was removed by treating with Lugol's solution and sodium thiosulphate.

The following stains were used employing standard staining techniques (Bancroft and Stevens, 1977).

Luxol fast blue . . . . . Myelin ----------blue/black

Silver methenamine. . . . . Neuroglia cells --------dark grey

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[1]A formulation of purified paraffin and synthetic polymer; Fisher Scientific Company, Fairlawn, New Jersey.

Silver methenamine.	.Collagen	---------- pale grey
Van Gieson.	.Collagen/osteoid	---------- red
	Other tissues	---------- yellow
P.A.S..	.Collagen	---------- pale pink
Hematoxylin and eosin (H&E) . . .	.Nuclei	---------- blue/black
	Cytoplasm	---------- shades of pink
	Muscle/collagen	-- pale pinkish red
Methylene blue.	.Nerve fibers	---------- blue

RESULTS

Retinal Vasculature

The retinal vasculature of 14 eyes from 14 sheep was examined during the course of this investigation while the animal was on prebypass, bypass, and postbypass phases (N1, B, N2). The ophthalmoscopic examination revealed no new vascularization of the fundus in any of the phases as the number of vessels crossing the optic disc were counted and compared against the fundic photographs taken during the corresponding phases. There was, however, evidence of moderate congestion of the retinal vessels, especially the main veins supplying the four quadrants of the retina (Figure 3). This enlargement or engorgement of blood vessels was confirmed by histologic sections which showed that, in 10 out of 22 slides examined, some vessels were observed to establish contact with the pigment epithelium (Figure 10). No comparative observations were made from animals that had the tracheal implants, but were not placed on bypass breathing (i.e., the controls). Cystic spaces, close to the ora serrata, were observed in both groups.

The outer diameters (O.D.) of the superotemporal retinal veins were measured from the fundic photographs taken during the three phases of this investigation (Table 2). The prebypass and bypass measurements: in 33 fundic photographs all the bypass measurements showed greater O.D. values than the prebypass values. Bypass and postbypass measurements: of the 32 pairs of fundic photographs used for comparison, 26 showed higher bypass values, while six showed no difference between the bypass and postbypass diameters of the superotemporal retinal vein.

Table 2. Retinal vasculature - outer diameter of the superotemporal retinal vein (μm)

Sheep No.	Phase[a]	(Slides) Trial No. 1	No. 2	No. 3
172	1	43	48	49
	2	72	74[b]	65[b]
	3	51	-[b]	-[b]
1728	1	50	49	54
	2	80	65	75[b]
	3	65	60	-[b]
195	1	50	56	46
	2	62	64	70
	3	60	43	53
197	1	62	63	65
	2	70	75	72
	3	70	75	70
1978	1	52	74	78
	2	68	85	80
	3	68	68	80
001	1	80	85	64
	2	95	90	80
	3	68	68	80
815	1	50	45	-[b]
	2	81	85	84[b]
	3	65	61	-[b]
120	1	48	63	-[b]
	2	64	66	-[b]
	3	62	52	-[b]
146	1	50	49	46
	2	70	58	67
	3	66	58	54

[a]Normal breathing (Phase 1 or N1); bypass breathing (Phase 2 or B); normal breathing (Phase 3 or N2).

[b]Not measured.

Table 2. *Continued*

Sheep No.	Phase[a]	(Slides) Trial No. 1	No. 2	No. 3
42	1	80	80	-[b]
	2	100	-[b]	89
	3	83	-[b]	82
105	1	45	46	44
	2	53	49	50
	3	43	45	-[b]
51	1	71	62	-[b]
	2	74	73	-[b]
	3	62	60	-[b]
84	1	55	65	-[b]
	2	62	70	72
	3	58	60	-[b]
80	1	35	-[b]	-[b]
	2	39	-[b]	-[b]
	3	30	-[b]	-[b]

Prebypass and postbypass measurements: thirty pairs of fundic photographs were compared. A total of 19 fundic photographs showed greater postbypass values, while 11 showed greater prebypass values (Figures 2, 3, 4).

In all sheep trials, the average outer diameter of the superotemporal retinal vein for the three phases (N1, N2, B) based on 103 observations was:

Treatments (Phases)	Mean diameter of the superotemporal retinal vein (μM)
N1	56.73
N2	59.79
B	70.45

The analysis of variance method was used to compare the means. F and t tests were carried out to determine the significance probabilities of the treatment (phase) mean differences. These probabilities are considered as the strength of the evidence against the hypothesis of no treatment differences. The calculated differences represent a true underlying difference, if the probability is less than 0.05.

Source	DF	ANOVA SS	MS	F-value	PR>F	Remarks
Sheep	13	6377.02	--	19.99	0.0001	Very significant
Treatment	2	1453.88	--	29.63	0.0001	Very significant
Error	26	--	24.54	--	--	

This result indicates that at least one significant difference exists between treatments (phases). Two t-tests were constructed to determine where the differences were among the treatments.

1) Does N1 differ from N2?

2) Does the average of N1 and N2 differ from B?

Question	t-value	Probability	Remarks
$N1 \neq N2$	1.63	$.1 < P < .2$	No statistical difference
$\frac{N1 + N2}{2} \neq B$	-7.52	$P < 0.001$	Definitely there is a difference

From the above statistical analysis, the superotemporal retinal vein diameter of the prebypass (N1) and postbypass (N2) values show no significant difference between them and during these two phases, the sheep was breathing normally through the nostrils. However, the average diameter of the vein during prebypass and postbypass breathing was significantly

different from the diameter of the same vein photographed during the by-pass breathing. This indicates that the diameter of the vein increased during bypass breathing and decreased as the animal was returned to normal breathing.

Electrical Activity of the Retina (ERG)

Two trials were carried out for each of the five sheep, except sheep #70 with only one trial (Table 3). In one animal serial tracings of the electroretinogram (ERG) were recorded during normal (N1) and bypass (B) breathing. The amplitude of the b-wave measured 1.2cm during normal (Figure 5) and 1.8 cm during bypass (Figure 6). The amplitude of the b-wave was higher in all animals during bypass (Figure 7), except in the last two recordings for sheep #121 where the normal breathing values were higher (Figure 8).

In all the animals the average of the b-wave amplitude for the two treatments (phases N and B) of each trial (based on 30 observations) was as follows:

Treatments (phases)	Amplitude of the b-wave (cm)
N	1.21
B	1.54

The analysis of variance procedure was carried out and the F-test statistics was used to compare the means of the observations during both normal and bypass breathing as follows:

Source	DF	ANOVA SS	M S	F-test	PR>F	Remarks
Sheep	4	0.25	-	8.23	0.033	Significant difference exists
Treatments	1	0.25	-	32.98	0.005	Very significant
Error	4	-	0.008	-	-	

This shows that a significant difference existed between the treatments (phases), i.e., the amplitude of the b-wave was significantly greater during bypass breathing.

A similar statistical method was used to compare the means of the duration of the b-wave (Table 4). The average of the means for the two treatments (phases) was as follows:

Treatments (phases)	Duration of the b-wave (cm)
N	0.392
B	0.316

Analysis of variance:

Source	DF	ANOVA SS	M S	F-test	PR>F	Remarks
Sheep	4	0.024	-	26.16	0.0039	Very significant
Treatments	1	0.014	-	2.77	0.0014	Very significant
Error	4	-	0.0002	-	-	

This indicates that the treatment differences over both trials were quite significant, i.e., a change occurred in the duration of the b-wave when the sheep was placed on bypass breathing.

Table 3. Electrical activity of the retina (ERG) - b-wave amplitude (cm)

Sheep No.	Phase[a]	Trial No. 1	Trial No. 2
39	1	2.1	1.2
	2	3.0	1.3
	3	1.2	1.05
	4	1.6	1.15
	5	0.8	1.0
	6	1.4	1.2
133	1	0.9	0.8
	2	1.6	1.15
	3	1.1	1.05
	4	1.6	1.30
	5	1.0	1.2
	6	1.2	1.25
31	1	1.1	1.8
	2	1.5	2.0
	3	1.2	1.4
	4	1.7	1.5
	5	1.7	1.0
	6	1.8	1.9
121	1	1.2	1.1
	2	1.4	1.4
	3	1.2	1.2
	4	1.35	1.1
	5	0.8	1.4
	6	0.9	1.35
70	1	1.2	-[b]
	2	1.3	-[b]
	3	1.5	-[b]
	4	2.2	-[b]
	5	1.3	-[b]
	6	1.7	-[b]

[a]1, 3, and 5 - Normal breathing; 2, 4, and 6 - bypass breathing.

[b]Trial not performed.

Table 4. Electrical activity of the retina - b-wave duration (cm)

Sheep No.	Phase[a]	Trial No. 1	Trial No. 2
39	1	0.8	0.5
	2	0.6	0.4
	3	0.35	0.49
	4	0.3	0.49
	5	0.4	0.3
	6	0.3	0.29
133	1	0.5	0.4
	2	0.4	0.25
	3	0.3	0.25
	4	0.25	0.2
	5	0.3	0.3
	6	0.2	0.3
31	1	0.35	0.4
	2	0.25	0.3
	3	0.3	0.45
	4	0.25	0.25
	5	0.4	0.4
	6	0.3	0.3
121	1	0.4	0.5
	2	0.25	0.4
	3	0.5	0.3
	4	0.4	0.35
	5	0.45	0.3
	6	0.4	0.35
70	1	0.4	-[b]
	2	0.35	-[b]
	3	0.35	-[b]
	4	0.25	-[b]
	5	0.3	-[b]
	6	0.25	-[b]

[a] 1, 3, and 5 - normal breathing; 2, 4, and 6 - bypass breathing.

[b] Trial not performed.

Blood Gas Analysis

The calculated data from the trials are tabulated (Table 5). The mean values for the respiratory rate (RR), hydrogen ion concentration (pH), arterial partial carbon dioxide tension (Pco_2), partial oxygen tension (Po_2) and total carbon dioxide concentration (Tco_2) were as follows:

Treatments (phases)	RR	pH	Pco_2	Po_2	Tco_2
N	19.77	7.33	54.52	99.42	29.37
B	19.47	7.35	54.45	99.24	29.94

Analysis of variance

Dependent variable: respiratory rate

Source	DF	ANOVA SS	M S	F-value	PR>F	Remarks
Sheep	4	32.60	-	1.46	0.2270	No difference
Treatments	1	1.35	-	0.24	0.6249	Definitely no difference
Error	53	-	5.58	-	-	

This analysis infers that there was no difference between the treatments (phases), i.e., there was no significant difference between the respiratory rate during both normal and bypass breathing.

Dependent variable: hydrogen ion concentration (pH)

Source	DF	ANOVA SS	M S	F-value	PR>F	Remarks
Sheep	4	0.25	-	13.88	0.0001	There is a difference
Treatments	1	0.005	-	1.11	0.2975	No difference exists
Error	53	-	0.0045	-	-	

Table 5. Blood gas analysis (Pco_2, Po_2, Tco_2 (mm Hg))

Sheep No.	Phase[a]	Trial									
		No. 1					No. 2				
		RR.	pH	Pco_2	Po_2	Tco_2[b]	RR.	pH	Pco_2	Po_2	Tco_2[b]
158	1	20	7.387	46.3	197.7	28.8	24	7.378	45.9	82.8	28.5
	2	18	7.371	46.7	188.7	28.7	23	7.446	47.2	82.5	29.2
	3	22	7.366	51.9	112.2	28.2	20	7.462	39.4	74.9	28.1
	4	20	7.368	48.2	98.9	30.6	18	7.482	37.8	83.2	28.1
	5	20	7.440	34.2	98.1	29.1	15	7.376	51.6	41.1	28.4
	6	18	7.492	37.9	78.2	32.0	15	7.342	49.1	42.9	29.2
316	1	20	7.038	112.0	80.6	32.7	24	7.220	48.8	115.0	21.4
	2	20	7.098	109.4	82.6	34.3	24	7.336	41.9	127.9	23.1
	3	18	7.159	85.6	49.3	33.7	22	7.330	40.7	154.8	22.1
	4	18	7.188	80.9	68.9	32.0	22	7.451	37.5	160.3	26.5
	5	18	7.223	70.9	103.0	30.6	24	7.338	49.7	112.6	25.9
	6	18	7.215	8.24	83.8	35.0	24	7.344	52.8	123.5	29.6
210	1	22	7.276	51.0	104.6	30.7	20	7.357	41.1	98.2	29.2
	2	22	7.201	52.1	101.2	29.5	20	7.340	43.0	96.9	29.8
	3	18	7.441	34.2	98.1	30.1	20	7.346	44.0	104.0	28.8
	4	18	7.371	36.4	85.4	30.4	20	7.348	41.9	106.8	28.7
	5	16	7.362	47.0	100.1	29.2	18	7.370	50.5	80.4	32.1
	6	16	7.370	50.1	92.2	30.4	18	7.396	49.1	91.2	31.6
214	1	20	7.351	49.9	100.1	26.4	24	7.471	45.6	120.0	29.6
	2	20	7.342	50.1	98.4	25.2	24	7.421	47.9	118.4	29.0
	3	16	7.362	47.0	98.2	30.6	22	7.455	40.6	110.6	29.7
	4	16	7.364	50.1	95.9	29.1	22	7.457	40.1	109.1	29.7
	5	16	7.401	38.9	74.1	29.9	20	7.377	51.2	115.2	30.1
	6	16	7.424	34.2	70.9	29.4	20	7.341	49.1	112.6	29.4
270	1	14	7.138	102.0	90.6	32.0	20	7.230	58.6	112.1	28.6
	2	14	7.198	99.6	93.0	34.1	20	7.347	51.9	121.3	27.0
	3	16	7.259	84.6	58.3	33.5	22	7.335	50.6	99.6	29.1
	4	16	7.288	82.9	68.9	33.7	22	7.451	49.5	100.2	28.0
	5	20	7.221	70.9	101.1	34.6	22	7.339	50.9	95.6	29.5
	6	20	7.214	81.4	94.9	35.1	22	7.346	52.4	98.4	29.8

[a] 1, 3, 5 = normal breathing; 2, 4, 6 = bypass breathing.

[b] millimoles per liter.

A significant difference in pH occurred between the sheep, but none between the treatments (phases), i.e., there was no difference in the pH during both normal and bypass breathing.

Dependent variable: partial carbon dioxide tension (Pco_2)

Source	DF	ANOVA S S	M S	F-value	PR>F	Remarks
Sheep	4	8062.65	-	11.35	0.0001	There is a difference
Treatments	1	0.067	-	0.00	0.9846	No difference
Error	53	-	177.62	-	-	

Again, the analysis shows that there was no significant difference in the Pco_2 during both normal and bypass breathing.

Dependent variable: Partial oxygen tension (Po_2)

Source	DF	ANOVA S S	M S	F-value	PR>F	Remarks
Sheep	4	868.94	-	0.26	0.90	No difference
Treatments	1	0.49	-	0.00	0.98	No difference
Error	53	-	840.31	-	-	

There was no significant difference in the Po_2 between normal and bypass breathing.

Dependent variable: Total carbon dioxide tension (Tco_2)

Source	DF	ANOVA S S	M S	F-value	PR>F	Remarks
Sheep	4	48.07	-	2.11	0.0931	No difference
Treatments	1	4.82	-	0.84	0.3624	No difference
Error	53	-	5.7	-	-	

Hence, the Tco_2 did not vary between the treatments (phases), i.e., it remained stable during both normal and bypass breathing.

Correlations were made between pH, Pco_2, Po_2 and RR (a = correlation coefficient; b = probability).

Comparison of total values		Remarks
RR/Pco_2	a) 0.297 b) 0.001	No significant correlation
pH/Pco_2	a) 0.861 b) 0.0001	Negative correlation
Po_2/Pco_2	a) 0.294 b) 0.022	No significant correlation

The above analysis indicated that there was a negative correlation between pH and Pco_2, i.e., an increase in Pco_2 resulted in a decrease in pH.

Intraocular Pressure (IOP) and Mean Systemic Arterial Pressure (MP)

Two complete trials were carried out on three animals and one each for the remaining two (Table 6). Figure 9 represents parts of two recordings of two trials for sheep #262. From the recordings, it would be evident that there was a decrease in the intraocular and mean systemic pressures (from IOP, 20.4; MP, 120 to IOP, 19.8; MP, 112.5) as the animal was changed to bypass breathing. A similar decrease in the above pressures was recorded for all the sheep, except one in which there was an increase on bypass in two recordings (Table 6).

Table 6. Intraocular (I.O.P.) and mean systemic pressure (MP); (mmHg)

Sheep No.	Phase[a]	Intraocular pressure	Mean pressure
262	1	20.4	120
	2	19.8	112.5
	3	20.1	115.0
	4	18.9	110
290	1	21.0	142.5
	2	19.1	130.0
	3	20.25	135.0
	4	19.1	105.0
105	1	17.00	102.5
	2	21.7	125.0
	3	-[b]	-[b]
	4	-[b]	-[b]
174	1	18.0	107.5
	2	16.0	100.0
	3	24.0	95.0
	4	20.0	90.0
47	1	17.6	36.0
	2	16.0	76.0
	3	-[b]	-[b]
	4	-[b]	-[b]

[a] 1, N1; 2, B1; 3, N2; 4, B2.

[b] Trial not performed.

The means for the IOP and MP (based on 16 observations) were as follows:

Treatments (Phases)	IOP (mmHg)	MP (mmHg)
N1	18.80	111.70
B1	18.52	109.70
N2	21.45	115.00
B2	19.33	101.67

Analysis of variance

Dependable variable: intraocular pressure

Source	DF	Type 1 SS	MS	F-value	PR>F	Remarks
Sheep	4	14.93	-	0.77	0.58	No difference
Treatments	3	12.31	-	0.84	0.51	No difference
Error	8	-	4.85	-	-	

This indicates that there was no statistical difference in the intraocular pressure during both normal and bypass breathing.

Dependable variable: mean systemic pressure

Source	DF	Type 1 SS	MS	F-value	PR>F	Remarks
Sheep	4	3548.56	-	10.28	0.0031	There is a difference
Treatments	3	609.09	-	2.35	0.1482	There is no difference

Hence, there was no difference in the mean systemic pressure during both normal and bypass breathing.

Histologic Examination

In addition to the engorged veins depressing the retinal layers up to the pigment epithelium, described previously, lateral displacement of the retina was observed in 10 out of 22 sections examined (Figure 11). Further, moderate to severe elevation of the optic disc was observed in 15 of the 22 eyes from animals that had been on bypass for at least six days (Figure 12). No comparable observations were made on sections from the 10 control animals (Figures 13 and 14a, b).

DISCUSSION

Krabill (1979) demonstrated that the upper respiratory bypass cannula is an effective way of interrupting normal nasal breathing in the sheep. He noted that breathing through the nostrils ceased when the "bypass insert" of the cannula was in place and resumed when the "flow-through insert" was reestablished (Figure 1). In addition, he stated that an increase in brain temperature (average 38.90°C) occurred when the sheep breathed through the tracheal bypass compared to an average of 38.53°C during normal nasal breathing. His findings in the sheep are in agreement with the observations of Kluger and D'Alecy (1975) in the rabbit, who reported an average increase of 0.31°C in brain temperature during bypass breathing. Similar observations have been reported by Baker et al. (1974) in the dog breathing through a tracheostomy and Young et al. (1976) in the sheep when they occluded the nostrils and noted an immediate increase in the temperature of the hypothalamus.

From the work of Krabill (1979), it can be logically assumed that the sheep also possesses two heat exchange systems similar to those in the dog (Magilton and Swift, 1967). In the sheep, therefore, the "bypass insert" functionally eliminates the "external heat exchange system" (between the nasal venous plexuses and the ambient air), resulting in the return of comparatively warm venous blood from the nasal area into the cavernous sinus. This, in turn, may reduce the efficiency of the countercurrent heat exchange in the "internal system" (between the warm venous blood from the nasal area and the arterial blood destined to supply the

brain) because of the absence of the normal cool venous blood from the nasal mucosa, affecting the normal temperature gradient between them.

The blood supply to the retina of the sheep is derived partly from the intracranial part of the internal carotid and the external ophthalmic arteries (Baldwin, 1964; Getty, 1975; Ahmed, 1977). Carlton and McKean (1977) observed that, in the pronghorn, the ophthalmic rete is embedded in a venous pool with the veins and arteries arranged to accommodate blood flow in opposite directions. Thus, they concluded that such an arrangement has the necessary anatomic requirements expected of a countercurrent heat exchanger. Concededly, since the ophthalmic plexus also receives cool venous blood from the nasal mucosa, alterations of the venous blood temperature will simultaneously affect the temperature of the blood in both the cavernous sinus and the ophthalmic plexus. In this investigation, it may be presumed that the increase in cerebral blood temperature reported by Krabill (1979) in the sheep will also reflect in the retinal blood temperature when the sheep was placed on bypass breathing. The significant importance of the relative temperature of the blood to the nervous tissue has been emphasized by Serota and Gerard (1938), Hales (1973), and Ganong (1975). Further, they stated that metabolic heat production is relatively higher in the brain of mammals than in other tissues during normal physiologic activity. This may indicate that the cool arterial blood to the brain and retina (ectopic portion of the brain) might be important in the removal of some of the metabolic heat produced in these structures. This could be very essential since the nervous tissue is prone to damage by hyperthermia (Minard and Copman, 1963; Burger and Fuhrman, 1964: Carithers and Seagrave, 1976).

During bypass breathing, in the sheep a moderate congestion of the blood vessels of the retina and an increase in the outer diameter of the superotemporal retinal vein were observed when compared to that of the prebypass and postbypass breathing. Further, there was a mild elevation of the optic disc which was not detected by direct ophthalmoscopic examination during bypass breathing. This agrees with the observations of Hayreh (1975) who claimed that mild elevations of the optic disc were difficult to diagnose by direct ophthalmoscopy. He further described the normal optic disc as one with no elevation above the level of the adjacent retina and lying on the same plane. It must be acknowledged, however, that there are considerable variations in the appearance of the optic nerve head, all of which may be "within physiologic limits". However, Wolff (1940) stated that Zenker's solution (the fixative used) was capable of causing elevation of the optic disc during tissue processing. This problem was overcome by comparing the sections made from the bypassed animals with those from the nonbypassed animals (i.e., the controls).

Forbes and Wolff (1928) noted a linear correlation between pial arteries and the cerebrospinal fluid pressure in primates. Besides, a correlation between increased intracranial pressure and cerebral vasodilatation was reported in humans (Risberg et al., 1969; Sawada and Tazaki, 1977). The communication between cranial and orbital subarachnoid spaces was demonstrated in primates by Tenner and Trockel (1968). In addition, Schaltenbrand and Putnam (1927) reported that the meninges of the central nervous system are freely permeable, which has been confirmed by Brightman and Reese (1969) and Waggenner (1964). Further, the free passages of materials from the cerebrospinal fluid into the optic nerve sheath, optic

nerve, and optic nerve head up to the intermediary tissue of Kuhnt were demonstrated by Rodriguez-Peralta (1963) in rabbits, cats, guinea pigs, and rhesus monkeys; Grayson and Laties (1971) in rats, and Tsukahara and Yamashita (1975) in mice. In addition, Krabill (1979) found, in sheep on bypass breathing, a positive correlation between increase in brain temperature and cerebrospinal fluid pressure. He attributed these changes to the displacement of the space in an immovable, nonexpansive cranial vault by an increase in the size of the vessels which, in turn, exert pressure on the cerebrospinal fluid; conversely, when cerebral vasoconstriction takes place, a decrease in cerebrospinal fluid pressure results. He assumed, therefore, that with an increase in temperature of the arterial blood during bypass breathing, there will be a concurrent increase in the arterial blood flow to the cerebral structures in an effort to lower brain temperature, which might result in an increase in intracranial pressure. This increase in pressure in an already compact and peripherally-limited cranial cavity would cause compression of the surrounding tissues. Therefore, it can be inferred that the increased cerebrospinal fluid pressure would be transmitted to the orbital subarachnoid space. Hayreh (1977) demonstrated the concurrent rise of optic nerve tissue pressure with an increase in cerebrospinal fluid pressure in rhesus monkeys. He, further, showed that increase in intracranial pressure would cause a corresponding increase of the cerebrospinal fluid pressure, which would consequently affect the optic nerve tissue pressure. The pressure exerted on the optic nerve axons by the increased optic nerve tissue pressure causes axoplasmic flow stasis, resulting in the swelling of the prelaminar area of the optic nerve head. It could be assumed that the optic disc

elevation observed, following six days of bypass breathing, would take the same course in response to increased cerebrospinal fluid pressure reported by Krabill (1979) in the sheep during bypass breathing. This also conforms with the findings of Hayreh (1964) in monkeys when he observed a latent period of one to five days for disc elevation following increased cerebrospinal fluid pressure. This latent period he attributed to the time required to build up the optic nerve tissue pressure in response to increased cerebrospinal fluid pressure or to slow process of axoplasmic fluid accumulation, which subsequently results in the swelling of the axons in the optic nerve head. Besides, he noted that sudden lowering of the cerebrospinal fluid pressure does not lead to an immediate resolution of the disc elevation. This latter observation, again, agrees with the findings of this investigation since the disc elevation persisted during the three days postbypass period, when the sheep was returned to normal nasal breathing. In this study, therefore, one can presume that the increased cerebrospinal fluid pressure observed in the sheep during bypass breathing (Krabill, 1979) might be responsible for the disc elevation and its persistence after three days of normal breathing (which will presumably lower the cerebrospinal fluid pressure). The pathogenesis of disc edema, following increased cerebrospinal fluid pressure, has been reported by Hayreh (1964, 1977) in monkeys.

The venous congestion observed during the bypass breathing is concordant with the observation made by Hayreh (1977) when he reported that vascular congestion was secondary to disc edema. This was attributed to the compression of the thin-walled central retinal vein by swollen axons as it passed through the optic nerve head.

The decreased intraocular pressure recorded during the bypass breathing was not statistically significant. However, changes in intraocular and optic nerve tissue pressure gradient have been implicated in the production of axoplasmic flow stasis (Rodriguez-Peralta, 1966; Ernest and Potts, 1968). These two pressures have been shown to meet around the area cribrosa in monkeys by Peyman and Apple (1972) and Minckler and Tso (1976a). Besides, Primrose (1974) and Hayreh (1977) have demonstrated in monkeys that the intra-axonic pressure (which is partly responsible for anterograde flow of axoplasm in the optic nerve fibers) depends on the intraocular and optic nerve tissue pressures, with the intraocular pressure usually higher in such an amount that the flow gradient is towards the retrolaminar area. An increase in the cerebrospinal fluid pressure, therefore, would increase the optic nerve tissue pressure resulting in a flow stasis due to the reversal of the pressure gradient. However, the five minutes of bypass recordings of the intraocular pressure are definitely inadequate for an appreciable rise in optic nerve tissue pressure or axoplasmic fluid accumulation to elicit a marked change in the intraocular pressure. However, Parker (1911) reported from experimental and clinical studies in humans that optic disc edema in raised intracranial pressure appeared first in eyes with very low intraocular pressures. This, however, is not to dispute the occurrence of disc edema in the presence of increased intracranial pressure, which Levy (1974) and Minckler and Tso (1976b) claimed could occur by a different mechanism.

The lower intraocular pressure recorded for one sheep (Table 6, #105) during the normal breathing was attributed to the pressure applied to the eyeball during the insertion of the needle prior to recording. The

intraocular pressure in this animal did not stabilize following the insertion of the needle in the anterior chamber of the eye. There was a continuous rise through the normal and bypass breathing phases. This was attributed to the very low depth of anaesthesia attained in this animal, which later died of shock.

The decrease in intraocular pressure was concurrent with a decrease in the mean systemic blood pressure during bypass breathing. However, Heymans (1921) in the dog, Rodbard (1948) in the chicken and cat, and Newman and Wolstencroft (1960) in dogs, cats and rabbits, have observed that warming the blood circulating through the brain, 3-5°C above normal body temperature, produced a sudden decrease in blood pressure leading to eventual death. However, the temperature increase reported by Krabill (1979) in the sheep during bypass breathing was only an average of 0.37°C. This comparatively small temperature increase in the blood circulating through the brain might be proportional to the relatively small decrease in the mean systemic blood pressure.

Statistical analysis of the amplitude of the b-wave of the electroretinogram (ERG) recordings during the normal and bypass breathing indicated a significant difference between these two phases (Figure 7). This observation is in conformity with the observations of Winkler (1972) in an isolated rat retina when he noticed changes in the amplitude of the components of the ERG as the temperature of the medium was altered. Similar techniques have been used by Tomita (1965, 1978) for intracellular recordings from individual retinal cells of cold and warm blooded animals. The increase in temperature of the blood to the retina is, therefore, comparable to increasing the temperature of the medium and

might be considered to have a similar effect on the components of the ERG (Winkler, 1972).

There were no statistical differences in partial carbon dioxide tension (Pco_2), hydrogen ion concentration (pH), and partial oxygen tension (Po_2) of arterial blood taken during normal and bypass breathing. From this finding, it is evident that the tracheal bypass had no effect on the dead air space of the respiratory tract and in no way influenced the Pco_2 and pH of the arterial blood. Winkler (1972) demonstrated, with isolated rat retina, that variations in Pco_2 and pH markedly and selectively affect the amplitude of the b-wave of the ERG.

Benda (1946) and Apgar (1970) described certain maldevelopments in the skull of mongoloids (Down's syndrome). The deficiency in the development of the facial bones has resulted in difficulty in breathing through the nostrils as evidenced by the fact that mongoloids are, to a great extent, mouth breathers. This facial modification compares favorably to the animal models used in this study during tracheal bypass breathing, since mouth breathing, as in mongoloids, would deprive the cavernous sinus of an important source of cool venous blood from the nasal area. Further, Benda (1946) observed the absence of diploe, normally found in flat bones of the skull, which indicates the absence of emissary veins. The emissary veins normally connect the veins of the scalp and dura. It can be inferred from these observations that mongoloids are deprived of the two mechanisms which appear to be functional in regulating brain temperature, i.e., the nasal mucosa and the scalp. In addition, Gardiner (1967), Williams et al. (1973), and Pesch and Nagy (1978), have described various visual abnormalities peculiar to mongoloids. For example, Williams et al. (1973)

reported that mongoloids have significantly more retinal vessels crossing the margin of the optic disc in an unusually spoke-like array. Though some retinal vascular changes were observed in this investigation, none was comparable to the pattern described for mongoloids. However, the limited period of bypass breathing (simulating the situation in mongoloids) may not have satisfied all the conditions in mongoloids, which is a chromosomal abnormality, more specifically, a triploidy of the twenty-first chromosome, resulting in a total of forty-seven chromosomes. On the other hand, since this abnormality generally occurs prior to fertilization of the egg by the sperm, one would expect that the mouth breathing in mongoloids is secondary to the malformation of the facial bones and not a direct effect of the genetic inbalance (Michejda and Menolascino, 1975). This pilot study, however, leaves some scope for further investigations.

SUMMARY

To evaluate the morphologic and functional changes that occurred in the eye when the sheep was placed on bypass breathing, the vascular pattern of the fundus, the electrical activity of the retina, the intraocular pressure of the eye, the arterial blood gas analysis, and histologic study of the retina and optic nerve head were carried out during both normal and bypass breathing. Thirty-eight sheep, ages ranging from eight weeks to five years, were used.

Fourteen eyes from 14 different sheep were examined ophthalmoscopically and fundic photographs taken during the prebypass, bypass, and postbypass phases. Generally, the fundic vasculature showed moderate congestion of the retinal veins during the bypass phase. The outer diameter of the superotemperal retinal vein measured during all three phases, mentioned above, showed a very significant difference between the average of the prebypass and postbypass values and the bypass value, indicating that changes occurred in the outer diameter of the vein as the sheep was placed on bypass.

The electroretinogram (ERG) was recorded from five animals during two trials, two days apart. During each trial, the ERG was recorded three times from each animal at 10 minute intervals during normal and bypass breathing. There was, however, a negative correlation between the amplitude of the b-wave and its duration.

The intraocular pressure (IOP) and mean systemic blood pressure (MP) were measured from five animals, each with two trials. Recordings showed

a slight decrease in both the IOP and MP during bypass breathing, which however, were statistically insignificant.

Arterial blood samples were taken from five animals in a similar set up to that of the ERG recordings. Their statistical analysis showed no significant difference in Pco_2, Po_2, Tco_2, respiratory rate, and pH during both normal and bypass breathing.

A total of 110 histologic sections from 22 animals that had been through six days of bypass breathing and 50 sections from 10 animals that had the tracheal implant but never were exposed to bypass breathing (controls) were used for this study. The bypassed animals showed variations from mild elevation of the optic disc, lateral displacement of the retina, marked congestion of the veins to extensive penetration of the retinal layers up to the pigment layer by engorged vessels.

No similarities to the vascular pattern described for mongoloids were observed, especially in terms of new vascularization or increased number of blood vessels crossing the optic disc margin during bypass breathing.

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APPENDIX

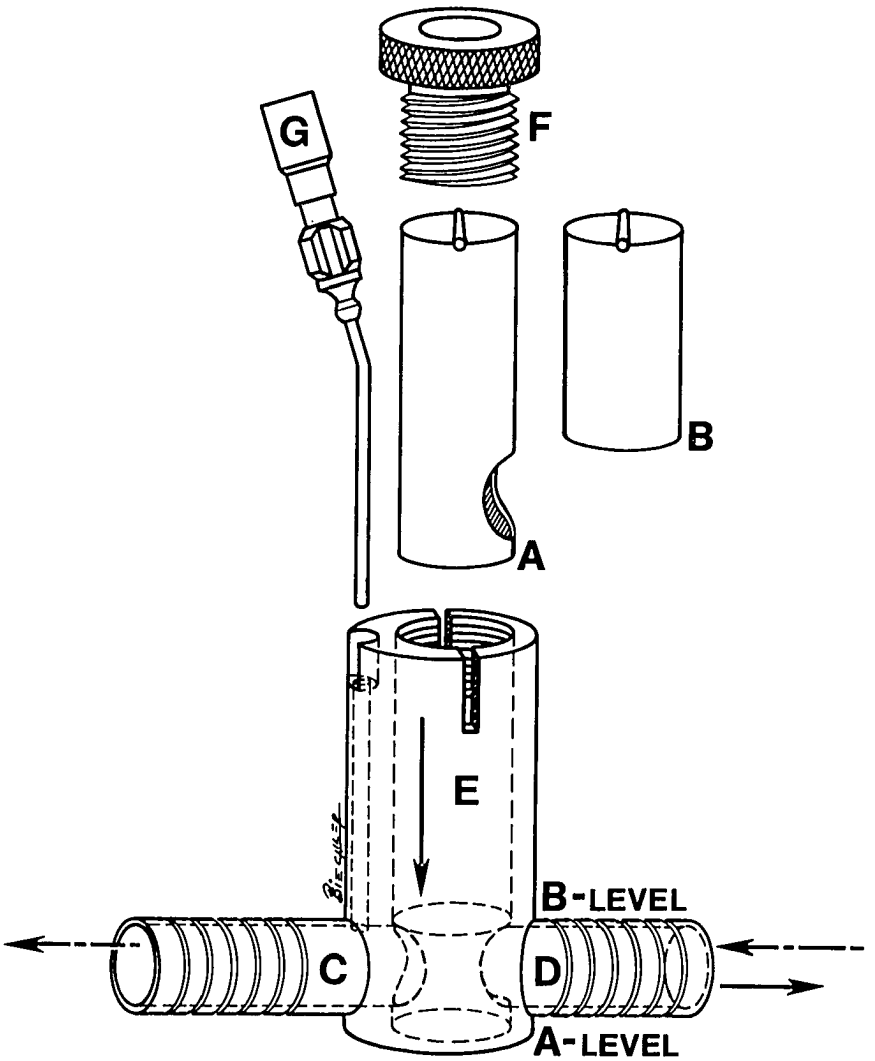


FIG.1 **UPPER RESPIRATORY(TRACHEAL) BYPASS CANULA (MODIFIED FROM KLUGER AND D'ALECY, 1975). SOLID ARROWS DENOTE BYPASS BREATHING WITH INSERT (A); BROKEN ARROWS DENOTE NORMAL BREATHING WITH INSERT (B).**

A - BYPASS INSERT
B - FLOW-THROUGH INSERT
C - CRANIAL TUBAL EXTENSION
D - CAUDAL TUBAL EXTENSION
E - BODY
F - CAP
G - LEUR LOCK NEEDLE WITH CAP

Figure 2. Fundic photograph of the area around the optic disc during the prebypass (N1) phase:

A Margin of the optic disc

B Superotemporal retinal vein

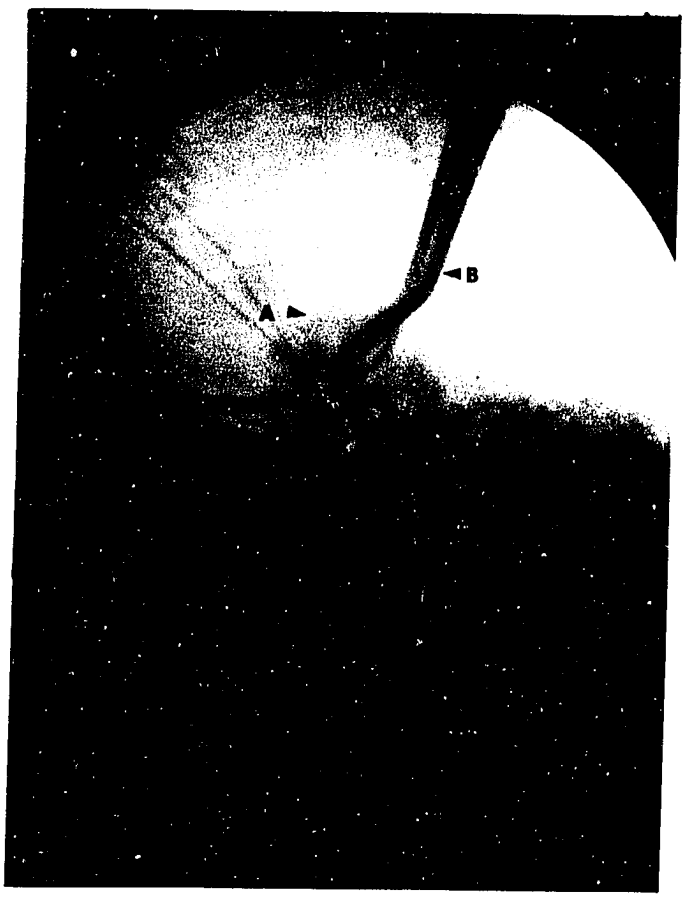

A
B

Figure 3. Fundic photograph of the area around the optic disc during bypass (B) phase showing mild congestion of the retinal vein

A Margin of the optic disc

B Superotemporal retinal vein

Figure 4. Fundic photograph of the area around the optic disc during the postbypass (N2) phase

A Margin of the optic disc

B Superotemporal retinal vein


B
A

Figure 5. Serial recordings of the electroretinogram (ERG) during normal breathing

H Amplitude of the b-wave

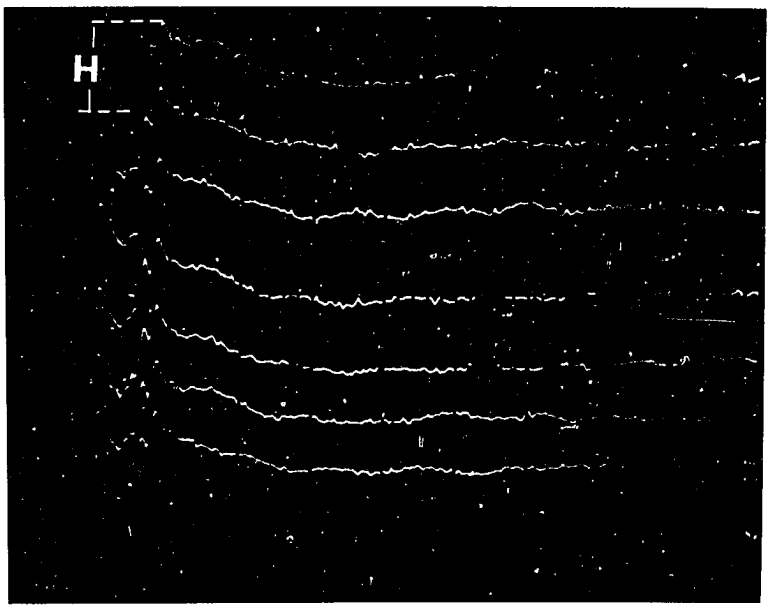

H

Figure 6. Serial recordings of the electroretinogram (ERG) during bypass breathing

H Amplitude of the b-wave

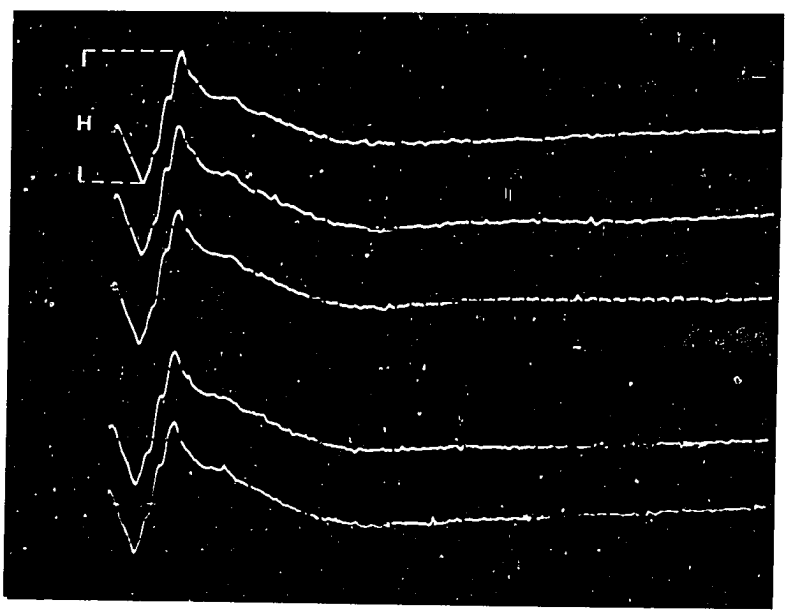

H
L

Figure 7. Electroretinogram recordings during normal and bypass breathing for one sheep

H Amplitude of the b-wave

D Duration of the b-wave

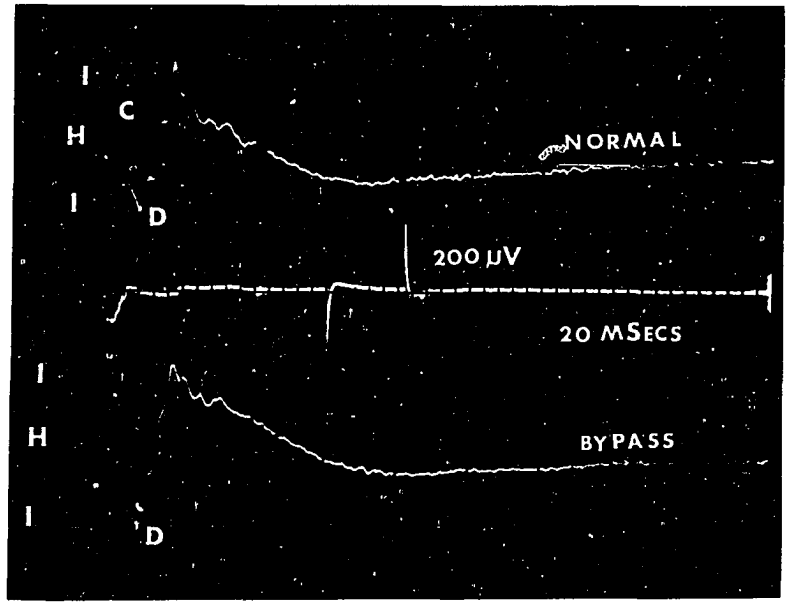
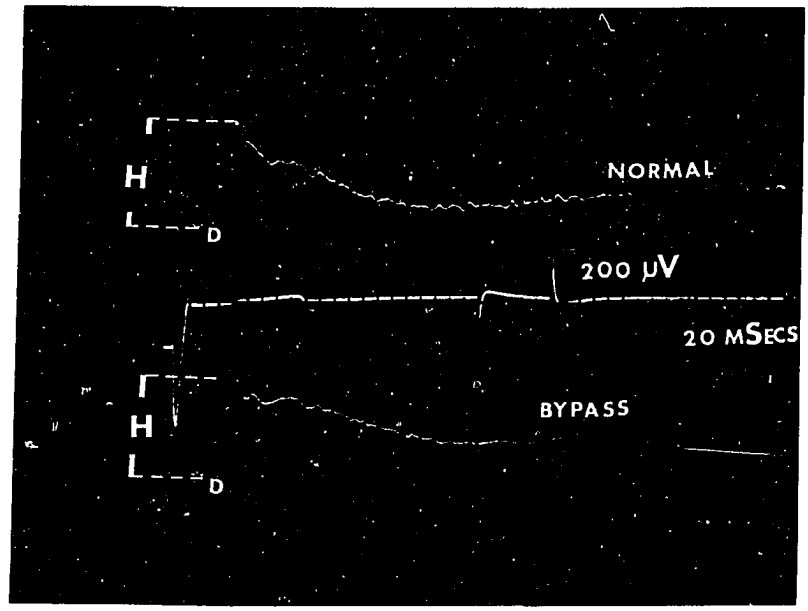

I
C
H
I
D
NORMAL
200 µV
20 MSECS
I
H
I
D
BYPASS

Figure 8. Electroretinogram recordings showing normal and bypass breathing with Leur lock needle cap open

H Amplitude of the b-wave

D Duration of the b-wave


H
L
D
NORMAL
200 μV
20 MSECS
H
L
D
BYPASS

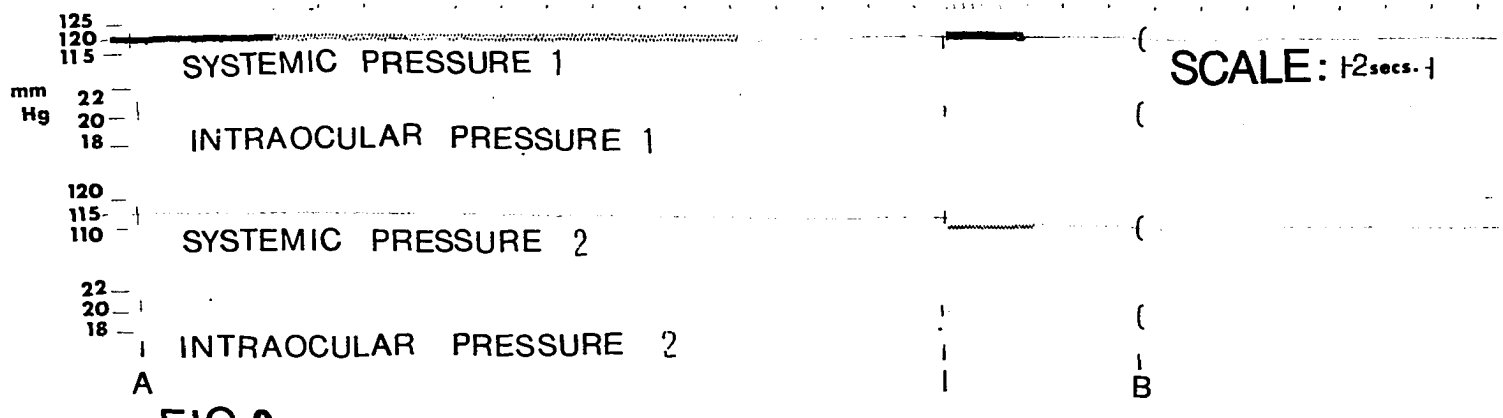


FIG.9 REPRESENTATIVE PARTS OF TWO TRIALS ON ONE SHEEP

A = on normal breathing

I = interval

B = on bypass breathing

Figure 10. Anteroposterior section of the retinal layers showing engorged vessel in contact with the pigment epithelium

A Nerve fiber layer

B Engorged blood vessel

C Inner nuclear layer

D Outer nuclear layer

E Pigment epithelium


B
C
D

Figure 11. Anteroposterior section of the optic nerve head and retina showing lateral displacement of the retina adjacent the optic nerve head

A Mild elevation of optic nerve head

B Lateral displacement of the retina

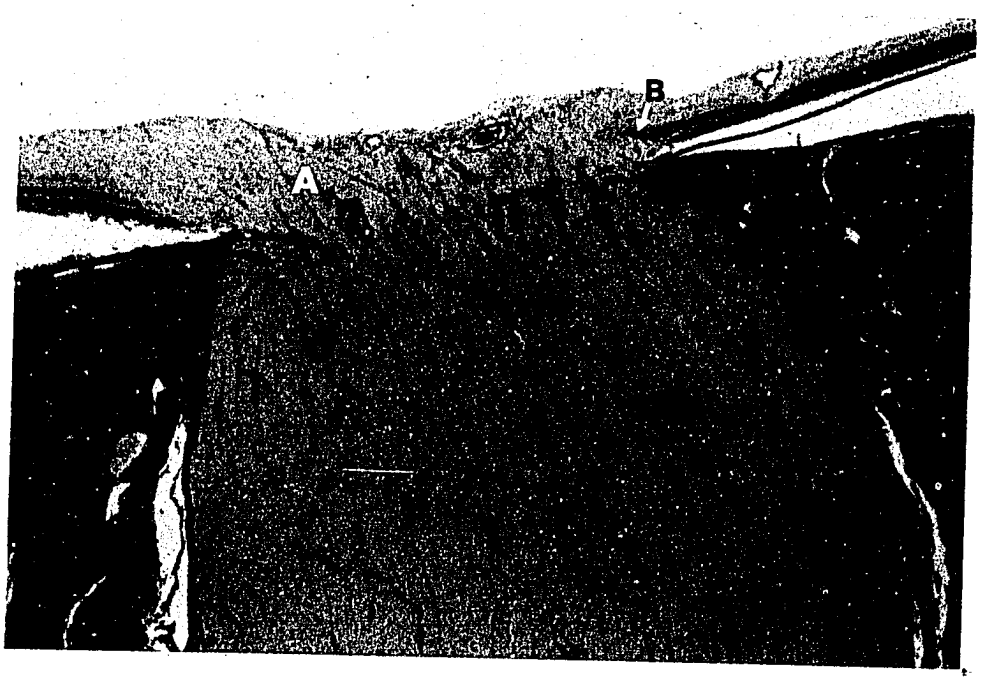

A
B

Figure 12. Anteroposterior section of the optic nerve head showing disc elevation

A Elevated optic nerve head

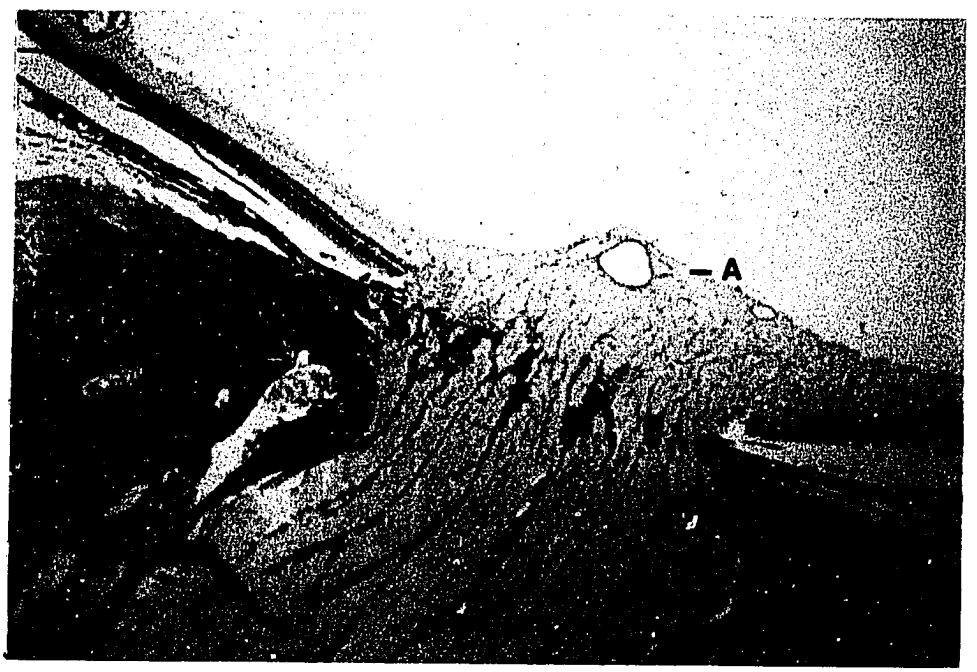

A

Figure 13. Anteroposterior section of the optic nerve head of a non-bypassed sheep with no disc elevation

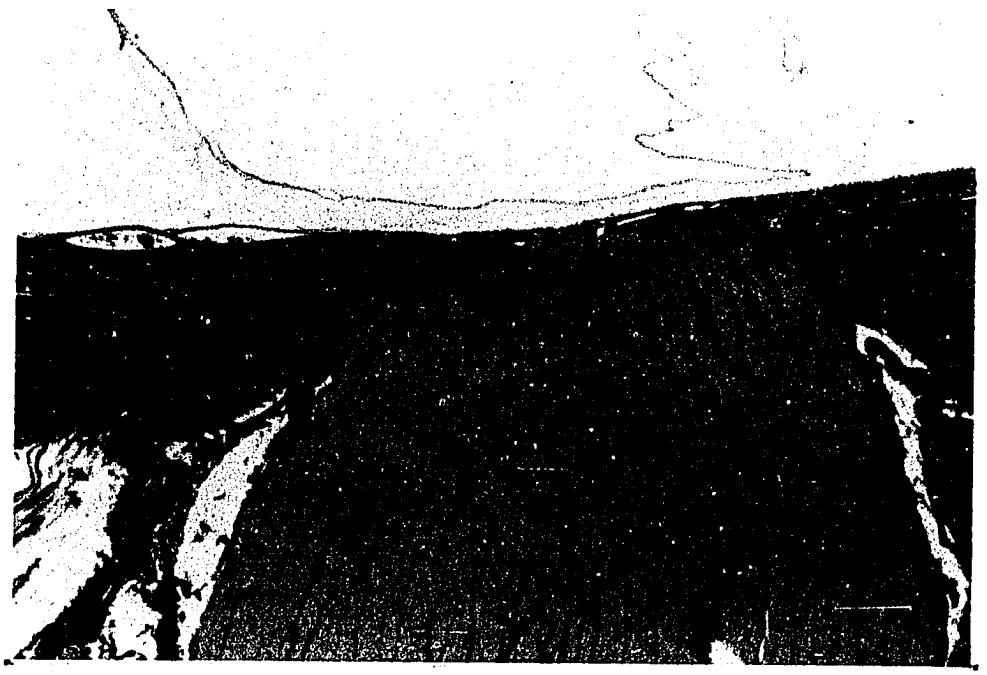


Figure 14a. Anteroposterior section of the retina in a nonbypassed sheep showing blood vessel in the nerve fiber layer

A Nerve fiber layer

B Blood vessel

C Inner nuclear layer

D Outer nuclear layer

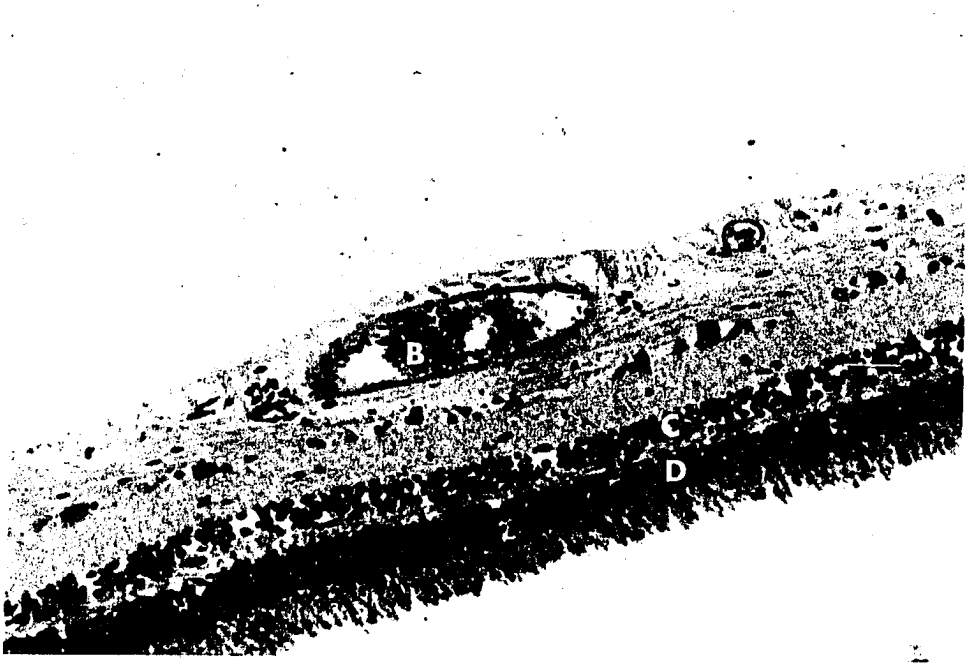

B
C
D

Figure 14b. Anteroposterior section of the optic nerve head of a non-bypassed lamb showing Bergmister's papilla

A Bergmister's papilla


A